PATENT COOPERATION TREATY

PCT

INTERNATIONAL PRELIMINARY REPORT ON PATENTABILITY
(Chapter II of the Patent Cooperation Treaty)

(PCT Article 36 and Rule 70)

Applicant's or agent's file reference	FOR FURTHER ACTION See Form PCT/IPEA/416	
E39080 ENI/J		
International application No.	International filing date *(day/month/year)*	Priority date *(day/month/year)*
PCT/NO2004/000397	22-12-2004	30-12-2003
International Patent Classification (IPC) or national classification and IPC		
See Supplemental Box		
Applicant		
Ullevål Universitetssykehus HF et al		

1. This report is the international preliminary examination report, established by this International Preliminary Examining Authority under Article 35 and transmitted to the applicant according to Article 36.
2. This REPORT consists of a total of 5 sheets, including this cover sheet.
3. This report is also accompanied by ANNEXES, comprising:
   a. ☒ *(sent to the applicant and to the International Bureau)* a total of 4 sheets, as follows:
      ☐ sheets of the description, claims and/or drawings which have been amended and are the basis of this report and/or sheets containing rectifications authorized by this Authority (see Rule 70.16 and Section 607 of the Administrative Instructions).
      ☐ sheets which supersede earlier sheets, but which this Authority considers contain an amendment that goes beyond the disclosure in the international application as filed, as indicated in item 4 of Box No. I and the Supplemental Box.
   b. ☐ *(sent to the International Bureau only)* a total of (indicate type and number of electronic carrier(s)) _____, containing a sequence listing and/or tables related thereto, in electronic form only, as indicated in the Supplemental Box Relating to Sequence Listing (see Section 802 of the Administrative Instructions).
4. This report contains indications relating to the following items:
   - ☒ Box No. I Basis of the report
   - ☐ Box No. II Priority
   - ☐ Box No. III Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
   - ☐ Box No. IV Lack of unity of invention
   - ☒ Box No. V Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement
   - ☐ Box No. VI Certain documents cited
   - ☐ Box No. VII Certain defects in the international application
   - ☐ Box No. VIII Certain observations on the international application

Date of submission of the demand	Date of completion of this report
08-06-2005	05-04-2006
Name and mailing address of the IPEA/SE Patent- och registreringsverket Box 5055 S-102 42 STOCKHOLM Facsimile No. +46 8 667 72 88	Authorized officer Patrick Andersson/MP Telephone No. +46 8 782 25 00

Supplemental Box

In case the space in any of the preceding boxes is not sufficient.
Continuation of: Cover sheet

International patent classification (IPC)

B60R 19/02 (2006.01)
B60R 19/34 (2006.01)

INTERNATIONAL PRELIMINARY REPORT ON PATENTABILITY

International application No.
PCT/NO2004/000397

Box No. I Basis of the report

1. With regard to the **language**, this report is based on:

☒ the international application in the language in which it was filed

☐ a translation of the international application into _____, which is the language of a translation furnished for the purposes of:

- ☐ international search (Rules 12.3(a) and 23.1(b))
- ☐ publication of the international application (Rule 12.4(a))
- ☐ international preliminary examination (Rules 55.2(a) and/or 55.3(a))

2. With regard to the **elements** of the international application, this report is based on *(replacement sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to this report):*

☐ the international application as originally filed/furnished

☒ the description:

pages 1-40 as originally filed/furnished
pages* _____ received by this Authority on _____
pages* _____ received by this Authority on _____

☒ the claims:

pages _____ as originally filed/furnished
pages* _____ as amended (together with any statement) under Article 19
pages* 2-5 received by this Authority on 25-01-2006
pages* _____ received by this Authority on _____

☒ the drawings:

pages 48-66 as originally filed/furnished
pages* _____ received by this Authority on _____
pages* _____ received by this Authority on _____

☒ a sequence listing and/or any related table(s) – see Supplemental Box Relating to Sequence Listing.

3. ☐ The amendments have resulted in the cancellation of:

- ☐ the description, pages _____
- ☐ the claims, Nos. _____
- ☐ the drawings, sheets/figs _____
- ☐ the sequence listing *(specify)*: _____
- ☐ any table(s) related to the sequence listing *(specify)*: _____

4. ☐ This report has been established as if (some of) the amendments annexed to this report and listed below had not been made, since they have been considered to go beyond the disclosure as filed, as indicated in the Supplemental Box (Rule 70.2(c)).

- ☐ the description, pages _____
- ☐ the claims, Nos. _____
- ☐ the drawings, sheets/figs _____
- ☐ the sequence listing *(specify)*: _____
- ☐ any table(s) related to the sequence listing *(specify)*: _____

* *If item 4 applies, some or all of those sheets may be marked "superseded."*

INTERNATIONAL PRELIMINARY REPORT ON PATENTABILITY

International application No.
PCT/NO2004/000397

Box No. V **Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement**

1. Statement

Novelty (N)	Claims	1-54	YES
	Claims		NO
Inventive step (IS)	Claims	1-32,44, 47, 51, 54	YES
	Claims	33-43, 45-46, 48-50, 52-53	NO
Industrial applicability (IA)	Claims	1-54	YES
	Claims		NO

2. Citations and explanations (Rule 70.7)

Reference is made to the following documents in the international search report:
D1: Ji Y et al., 2001,
D2: Sohal DS et al.
D3: Gaussin et al.
D4: Rossant et al.

Document D1 is considered to represent the closest prior art. D1 describes an animal model that is heterozygotic regarding SERCA2, i.e. it expresses a single copy of SERCA2 whereas the other copy carries a null mutation. The effect of the mutation is studied with regard to heart function. It is clear from D1 that it would be desirable to study Ca2+ homeostasis in heart tissue without any SERCA2 expression. It is pointed out in D1 that complete loss of SERCA2 function in homozygous animals is embryonic lethal.

The present application seems to avoid this problem by producing rodents that post-natally are induced to become homozygotic. This is achieved by using a system inducible by tamoxifen see D2. It is surprising that the tamoxifen induced system works to study heart failure.

Therefore, claims 1-32 are considered novel, inventive and to have industrial applicability.

The invention according to claims 33-40 differs from D1 by using a Serca ATPase gene which is flanked by inserted recombination sites. This enables production of rodents that are hetero- or homozygotic regarding Serca ATPases.

.../...

Supplemental Box

In case **the space in any of the preceding boxes is not sufficient.**
Continuation of: Box V

The objective technical problem of the present claims is to provide an alternative method to produce Serca ATPase heterozygotic or homozygotic rodents.

The present application uses a system with heart specific tamoxifen induced recombination, this system is considered non-obvious. However, claims 33-40 are not limited to homozygotic rodents or heart specific expression using tamoxifen-inducible Cre Protein. To use inserted recombination sites in order to produce hetero- or homozygotes is a well established technique, see D3 or D4. Therefore, claims 33-40 lack an inventive step.

It is clear from D1 that it would be desirable to study Ca2+ homeostasis in heart tissue without any SERCA2 expression. The methods of claims 41-54 achieve the desires of D1. These methods are considered non-obvious in view of D1 unless provided with non-trivial technical means. Only claims 44, 47, 51 and 54 provides such technical means i.e. rodents according to previous claims. Hence, claims 33-43, 45-46, 48-50, 52-53 lack an inventive step.

The invention according to claims 44, 47, 51 and 54 is regarded novel, inventive and to have industrial applicability.

1.
A genetically modified rodent all of whose cells comprise a Serca ATPase gene modified by inserted recombination sites, the modification being homozygous.

2.
The rodent of claim 1 comprising several copies of the modified Serca ATPase gene.

3.
The rodent of claim 1, wherein the Serca ATPase gene is a Serca2 ATPase gene.

4.
The rodent of claim 1, wherein the recombination sites are of heterogenous origin.

5.
The rodent of claim 4, wherein the heterogenous recombination sites are of non-mammalian origin.

6.
The rodent of claim 5, wherein the recombination sites comprise loxP recombination sites.

7.
The rodent of claim 1 all of whose cells further comprise a gene encoding a heterogenous recombinase.

8.
The rodent of claim 7, wherein the heterogenous recombinase is of non-mammalian origin.

9.
The rodent of claim 8, wherein the recombinase is a Cre recombinase.

10.
The rodent of claim 7, wherein expression of the recombinase encoding gene is controlled by a regulatory nucleic acid sequence.

11.
The rodent of claim 10, wherein the regulatory nucleic acid sequence is inducible.

12.
The rodent of claim 11, wherein said regulatory nucleic acid sequence is inducible by tamoxifen.

13.
The rodent of claim 7, wherein expression of the recombinase gene is tissue-specific.

14.
The rodent of claim 13, wherein expression of the recombinase gene occurs in heart tissue.

15.
The rodent of claim 1, wherein the rodent is a mouse.

16.
A eukaryotic cell comprising a Serca ATPase gene modified by inserted recombination sites, the modification being homozygous.

17.
The cell of claim 16 comprising several copies of the modified Serca ATPase gene.

18.
The cell of claim 16, wherein the Serca ATPase gene is a Serca2 ATPase gene.

19.
The cell of claim 16, wherein the recombination sites are of heterogenous origin.

20.
The cell of claim 19, wherein the heterogenous recombination sites are of non-mammalian origin.

21.
The cell of claim 20, wherein the recombination sites comprise loxP recombination sites.

22.
The cell of claim 16 further comprising a gene encoding a heterogenous recombinase.

23.
The cell of claim 22, wherein the heterogenous recombinase is of non-mammalian origin.

24.
The cell of claim 23, wherein the recombinase is a Cre recombinase.

25.
The cell of claim 22, wherein expression of the recombinase encoding gene is controlled by a regulatory nucleic acid sequence.

26.
The cell of claim 25, wherein the regulatory nucleic acid sequence is inducible.

27.
The cell of claim 16, wherein the cell is of mammalian origin.

28.
The cell of claim 27, wherein the cell is of non-human mammalian origin.

29.
The cell of claim 28, wherein the cell is of rodent origin.

30.
The cell of claim 39, wherein the cell is of mouse origin.

31.
The cell of anyone of claims 16-30, wherein said cell is an embryonic cell.

32.
The cell of anyone of claims 16-30, wherein said cell is a cardiomyocyte.

33.
A gene encoding a Serca ATPase modified by inserted recombination sites.

34.
The gene of claim 33, wherein the Serca ATPase is a Serca2 ATPase

35.
The gene of claim 33, wherein the recombination sites are of heterogenous origin.

36.
The gene of claim 35, wherein the heterogenous recombination sites are of non-mammalian origin.

37.
The gene of claim 36, wherein the recombination sites comprise loxP recombination sites.

38.
The gene of claims 34, wherein said gene is substantially modified as set forth in SEQ ID 1.

39.
A vector comprising the gene of claim 33.

40
The vector of claim 39, wherein the vector is based on pBluescript II KS.

41.
A method for inducing defective Ca^{2+} handling in a non-human vertebrate, comprising the steps of inducing recombination and inactivation of a Serca ATPase gene.

42.
The method of claim 41, wherein the Serca ATPase gene is a Serca2 ATPase gene.

43.
The method of claim 41, wherein the Serca gene is inactivated in heart tissue.

44.
The method of claim 41, wherein said non-human vertebrate is the rodent of anyone of claims 7, 8, 9, 10, 11, 12, or 13.

45.
A method for inducing heart failure in non-human vertebrate, comprising the steps of inducing recombination and inactivation of a Serca ATPase gene in heart tissue.

46.
The method of claim 45, wherein the Serca ATPase gene is a Serca2 ATPase gene.

47.
The method of claim 45, wherein said vertebrate is the rodent of claim 14.

48.
A method for screening a compound or a mixture of compounds for activity against defective Ca^{2+} handling, comprising the steps of inducing recombination and inactivation of a Serca ATPase gene in a non-human vertebrate; administrating the compound or mixture to said mammal before and/or after the induced inactivation of the Serca ATPase gene.

49.
The method of claim 48, wherein the Serca ATPase gene is a Serca2 ATPase gene.

50.
The method of claim 48, wherein the Serca gene is inactivated in heart tissue.

51.
The method of claim 48, wherein said vertebrate is the rodent of anyone of claims 7, 8, 9, 10, 11, 12, or 13.

52.
A method for screening a compound or a mixture of compounds for activity against heart failure, comprising the steps of inducing recombination and inactivation of a Serca ATPase gene in heart tissue of a non-human vertebrate; administrating the compound or mixture to said mammal before and/or after the induced inactivation of the Serca ATPase gene.

53.
The method of claim 52, wherein the Serca ATPase gene is a Serca2 ATPase gene.

54.
The method of claim 52, wherein said vertebrate is the rodent of claim 14.